

The University of Chicago

ON THE RELATIONSHIP OF
THE THYROID GLAND TO REPRO-
DUCTION IN THE RAT AND
THE GUINEA PIG

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1936

By

RENATE MARTHA ROSS

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CHAPTER I

INTRODUCTION¹

In the study of factors that operate in the regulation of the reproductive system organs of internal secretion other than the gonads have been found to play a significant role. The work of Smith and Engle (1927) and Zondek and Aschheim (1927), though preceded by many clinical observations of importance and some experimental work, brought out clearly for the first time the importance of the pituitary gland in the regulation of the state and function of the gonads. In this laboratory, Moore and his associates for some time have sought additional information in regard to the factors that operate in the control of the normal course of events in reproduction. They have discussed a pituitary-gonad relationship both for animals that breed more or less continuously, as the rat or guinea pig, and for those whose reproductive processes are so regulated as to be of a strictly annual type (Moore and Price, 1932; Moore, Simmons, Wells, Zalesky and Nelson, 1934).

The work of Allen (1917) and Smith (1930) and scores of other investigators have revealed that hypophysectomy not only leads to involution of the gonads but that it causes atrophy of the thyroids and adrenals as well. A suggestive relation between the thyroid and pituitary was pointed out as early as 1888 by

¹This investigation has been aided by a Biological Research Grant from the Rockefeller Foundation to the University of Chicago.

Rogowitsch who noted enlargement of pituitaries in thyroidectomized dogs and rabbits. Histologically all elements of the anterior hypophysis become hypertrophic and cell changes are quite similar to those produced by gonadectomy; the eosinophil granules disappear (Hohlweg and Junkman, 1933; Severinghaus, Smelser and Clark, 1934). Physiologically, however, pituitaries from castrates and thyroidectomized animals are different in sex stimulating potencies. The report of Evans and Simpson (1930) has been questioned by Smith and Engle (1930), Van Horn (1933) and Van Dyke (1934) who found that thyroid removal does not increase the gonad stimulating capacity of the anterior lobe of the hypophysis. On the other hand, thyroid feeding is claimed by Van Horn (1934) to increase the gonad stimulating power of the pituitary. He explains this as a metabolic phenomena that speeds the rate of elimination of gonad hormone from the organism. That the hypophysis exerts a stimulating influence upon the thyroid was demonstrated in 1929 by Loeb and Aron independently. This pituitary thyrotropic factor has been shown by Loeser (1931), Anderson and Collip (1933) and others to act independently from other pituitary hormones.

In the light of a pituitary-gonad and pituitary-thyroid relationship, the demonstration of a possible thyroid-gonad relationship would be of special interest. Indications for such a relationship have long been recognized clinically in thyroid enlargement during puberty, menstruation and pregnancy and disturbed reproductive function during thyroid disturbances. This might suggest an influence of gonadal states on thyroid function, or the causes basic to gonadal changes might likewise be operating on the thyroid. Many experiments have been conducted to study the response of the reproductive system to hypo- and hyperthyroidism. On the whole, however, there has been a decided lack of

consistency in results so far observed and much remains to be learned regarding the specific role played by the thyroid gland in reproductive phenomena.

The present paper constitutes a report of observations made in an attempt to study the importance of the thyroid gland in reproduction in the laboratory rodents such as the rat and the guinea pig. Both the effects of gonadectomy on the state of the thyroid and its function and the influence of modified thyroid conditions on the gonads, has constituted the main object of this study.

This problem was suggested by Dr. Carl R. Moore and I am only too glad to make acknowledgments for facilities to carry on the work and for unceasing interest during the study.

CHAPTER II

THE INFLUENCE OF THE GONADS ON THE THYROID

A. Literature

The question of a possible influence of the gonads on the thyroid can be studied either from a physiological or an anatomical approach. The influence of the gonadal hormones on the physiological activity of the thyroid is expressed by the basal metabolism and has been studied by several investigators in various species of mammals. Riddle surveys the work in this field in Sex and Internal Secretion and as yet no conclusive results have been obtained. The majority of the data suggest that gonadectomy diminishes the metabolic rate, but as to how far this is due to the influence of the gonads on the thyroid or on the adrenals is as yet unknown. The clinical use of Antuitrin S on hyperthyroid patients in active ovarian stages to lower the basal metabolism suggests that an overproduction of theelin inhibits the thyroid (Starr and Patton, 1934).

The histological study of the thyroid in woman during various stages of reproductive activity furnishes the bulk of the evidence for a thyroid-gonadal relationship. This evidence indicates that puberty, menstruation, pregnancy, lactation, and, according to some authors, menopause are associated with an histologically demonstrable greater excretory activity in the thyroid gland. However, it has been observed in guinea pigs (Loeb, 1931) and dog (Jannsen and Löser, 1932) that human pregnancy urine has no effect on the thyroid. Injections of

folliculin into rats have produced depressed thyroid conditions (Bialet-Laprida, 1933) which the author explains as thyroid-ovarian antagonism.

Gonadectomy has been frequently employed in studying the problem of an influence of the gonads on the thyroid. Anderson and Kennedy (1933) gonadectomized mature male and female rats. After 50 days these animals exhibited a slight decrease in thyroid weight and histological changes which the authors interpreted as different stages of thyroid atrophy. Korenchevsky and Dennison (1934) also found slight decrease of thyroid weight in castrated rats. Using guinea pigs Loeb and Kippen (1936) found an increase in epithelial proliferation shortly following gonadectomy, but this stimulation ceased after several weeks and gave way to degenerative changes in the thyroids of some of these animals.

In my investigation guinea pigs and rats were used. The value of the guinea pig as an experimental animal lies in the sensitivity of its thyroid to minimal stimulations; whereas the rat can be gonadectomized at a period of earlier development and be more adequately controlled by littermates.

In order to study the effects of the gonads on the thyroid gland male and female guinea pigs, and male and female rats were gonadectomized and killed at various ages during their adult life. Mature experimental cryptorchid guinea pigs were used to show whether spermatogenic activity of the testis had any influence on the thyroid. Male and female rats were gonadectomized also postpuberally for the purpose of ascertaining the importance of the stage of development on the gonads at the time of operation.

B. Gonadectomized Guinea Pigs

a. Males.--To determine whether castration produces positive demonstrable effects on the thyroid gland of the male guinea pig, 25 animals were operated at ages from four to six weeks after birth when the average body weight was approximately 200 grams. These were sacrificed when 18 months of age along with normal control males of similar ages. From the 25 operated males, 16 survived for autopsy to be compared with 10 normal controls. The recorded data of Table I presents a brief summary of these results.

The average weight of the thyroid glands of 15 males castrated for seventeen months was 169 milligrams as against an average of 123 milligrams for the 10 normal similar aged controls. This appreciable difference does not seem important considering the great variability in thyroid weight in normal animals. The data from the castrates include an animal, No. 42, which had an exceptionally heavy thyroid of 400 milligrams which is more than double the weight of any other of the fifteen castrate animals in this group. So if there is an increase in thyroid weight after castration it cannot be very clearly demonstrated and does not seem significant.

The average body weight of the castrated males at autopsy was 815 grams as compared with 732 grams of the controls. This represents an increase of 11 per cent in the experimental animals.

The results obtained from a group of 10 unilateral cryptorchid and unilateral castrate males are also summarized in Table I. They were included in this experiment to see whether or not spermatogenetic activity of the testes had any influence on the thyroid. They had been operated for two years and were older than the castrate and normal control groups.

TABLE I

Animal Number	Body Weight Grams	Thyroid Weight Milligrams	Pituitary Milligrams	State of Thyroid
A. Normal Male Guinea Pigs, 18 months of age				
72....	640	114	15	I, II
76....	590	112	16	I, II
90....	732	123	15.5	II, III
100....	776	143	15.2	II, III
101....	782	108	14.9	I, II, III
102....	772	138	15	II, III
103....	800	120	14.8	..
104....	784	126	15.2	..
105....	695	111	15.1	II, III
106....	754	139	15.6	II, III
Average	732	123	15.2	
B. Castrated Male Guinea Pigs, 18 months of age (Castrated 4-6 weeks of age)				
38....	1050	195	14	II, III
40....	948	148	13	II, III, IV
41....	1000	141	16	II, III
42....	960	400	19	I, II
47....	968	162	17	II, III, IV
49....	863	143	16	II, III
63....	800	143	18	II, III, IV
64....	762	161	16	II, III
70....	710	175	17	II, III
71....	681	32	15	II
73....	685	201	18	II
77....	590	125	16	II, III
79....	720	142	12	II, III
80....	670	108	13	III
Average	815	169	15.6	
C. Mature Male Guinea Pigs, two years after unilateral castration and unilateral experimental cryptorchidism (Castrated 4-6 weeks of age)				
23....	916	153	..	I
24....	1004	96	..	II, III
26....	860	108	..	II,
27....	616	123	..	II
29....	674	150	..	II, III
30....	800	154	..	III
31....	751	121	..	II, III
32....	850	280	..	I, II
34....	856	148	..	II
46....	625	126	..	II, III
Average	795	146	..	

The average thyroid weight of 146 milligrams is definitely within the range of the normal and castrate animals killed at 18 months of age. The average body weight at autopsy was 795 grams which is between the weight of the castrate and the controls.

Microscopic preparations of the seminal vesicle of the castrate showed that no gonad hormone had been present whereas the seminal vesicles of the cryptorchids were fully developed in almost complete absence of seminiferous tubules.

The difference between the average weight of the pituitaries of the castrates and the controls was negligible.

We can conclude from this experiment that castration in the male guinea pig does produce a very slight increase in the weight of the thyroid and a slight but more consistent increase in the general body weight.

b. Females.--Twenty-five female guinea pigs of approximately 200 grams body weight were spayed between four and six weeks of age and killed after 17 months. Twelve corresponding controls were sacrificed at the same time. Table II summarizes the results obtained at the autopsies of these animals. The weight of the thyroid remained unchanged by seventeen months castration. It ranged from 100 to 200 milligrams in both groups, averaging about 155 milligrams. The group of experimental animals contained an animal, No. 35, whose thyroid weighed 450 milligrams. This is the heaviest thyroid so far seen in this colony and shifted the thyroid weight average slightly in favor of the experimental animals.

At autopsy the average body weight of the spayed females was 840 grams as compared with the average of 783 grams for the controls. This represents a body weight increase of the gonadectomized animals of 8 per cent. The pituitary weights were

TABLE II

Animal Number	Body Weight Grams	Thyroid Weight Milligrams	Pituitary Milligrams	State of Thyroid
A. Normal Female Guinea Pigs, 18 months of age				
54.....	1023	149	22	II, III
58.....	880	125	18	I, II
59.....	804	162	18	II, III
60.....	812	189	17	II, III
78.....	780	180	16	II, III, IV
83.....	820	134	17	II, III
84.....	678	143	16	II, III
85.....	803	176	17	II
86.....	682	145	16	I, II
87.....	830	139	14	II, III
88.....	610	162	11	II
89.....	673	150	12	II
Average	783	155	16.2	
B. Castrated Female Guinea Pigs, 18 months of age (Castrated 4-6 weeks of age)				
37.....	950	210	..	II, III
39.....	1049	450	..	IV
43.....	722	142	16	II, III
44.....	1031	143	15	II, III
45.....	1030	138	20	II, III
48.....	960	142	18	II, III, IV
50.....	860	150	17	II, III, IV
51.....	882	134	18	II
52.....	800	180	21	II, III
53.....	880	155	17	II, III, IV
55.....	988	168	18	II, III, IV
56.....	851	97	18	I, II, III
57.....	860	175	24	II
61.....	840	191	17	II, III, IV
62.....	794	200	16	I, II, III
65.....	851	172	15	I, III
66.....	755	134	17	II
67.....	620	75	9	II, III
74.....	480	112	12	I, II, III
75.....	748	124	16	III, IV
81.....	780	119	12	II, III
82.....	722	142	16	III, IV, V
Average	840	162	16.5	

the same in both groups, averaging 16.5 and 16.2 milligrams, respectively. The spayed animals were all totally gonadectomized as indicated by the atrophied uteri. The control females had all been killed in dioestrus so as not to complicate their thyroid picture by the possible influence that the sexual cycle may have on the thyroid of the female guinea pig.

c. Histology of the thyroids of gonadectomized guinea pigs.--The thyroid of castrate, cryptorchid-castrate, spayed and normal guinea pigs of both sexes proved to be so similar histologically that their common discussion is justified.

One thyroid of each animal was fixed in Bouin's fixative and the other in formalin-Zenker (2.5 gr. potassium dichromate and 5.0 gr. mercuric chloride per 100 cc. water, 9 parts stock solution 1 part neutral formaldehyde). Serial sections were made of all thyroids fixed in Bouin's fluid. About thirty sections of different levels were mounted and stained with Hemotoxylin-eosin. In addition the Zenker fixed thyroids of some twenty of these animals were stained with Mallory triple stain (Krichevsky's modification, 1931).

A short review of the histology of the normal guinea pig will serve to recall the possible modifications produced by the absence of the gonadal hormone. The structural unit of the thyroid is the follicle, the size and shape of which changes in the course of individual development from small round follicles in young animals to more irregularly shaped larger follicles in older animals. A thyroid has larger follicles towards the periphery and smaller and more regular alveoli in the center. The inter-follicular spaces contain mainly a vascular connective tissue frame-work. The lumen of the follicle is filled with varying amounts of colloid in an actively secreting thyroid, whereas

under the stimulus of the thyrotropic hormone the colloid is in a stage of absorption. This state is manifested by bubble like vacuoles at the edges of the colloid masses. During strong thyroid hypertrophy these vacuoles are so large and numerous that the colloid has almost disappeared from the lumen. Colloid in absorption stages is thin and stains pale red with eosin. Hard, dark red colloid retracted from the edges is in resting thyroid stages. The follicles are lined inside by a single layer of epithelial cells varying from tall columnar to flat. The flattened appearance of the epithelium may be associated with thyroid atrophy, in which case the lumen is often filled with debris. The epithelium might also be flattened secondarily by pressure from abundant dense colloid. The cytoplasm of the epithelial cells may vary in consistency. This is indicated by a large and clear or darker and more granular appearance of the cytoplasm. The majority of the nuclei seen are large and round and mitosis may be present to a varying extent. Small numbers of condensed pyknotic, or atypically shaped nuclei are to be found normally. This condition can be considered pathological only if larger amounts of such abnormal nuclei are seen. Interfollicular cells are sometimes present but their significance is not yet clear.

Gonadectomy might influence the following elements of the histological picture of the thyroid.

1. Size and shape of follicles
2. Amount of connective tissue
3. Amount and consistency of colloid
4. Size and shape of the epithelial cells
5. Consistency of the cytoplasm
6. Structure of the nucleus
7. Amount of the interfollicular cells seen

Sections of thyroid tissue were examined with the above points in mind for the purpose of discovering thyroid characteristics peculiar to the sex or state of the reproductive system of the animal from which the gland was taken. No consistent correlations between the histological picture and the sex of the guinea pig could be detected. The thyroids of all mature guinea pig males and females, operated and controls, may be in any state of thyroid activity. The question remains whether or not there is a preponderance of certain thyroid states in the experimental and control groups. Loeb's method of counting mitoses seemed unsatisfactory in animals gonadless for long periods of time because any stimulating effect that may be observed directly after gonadectomy might disappear after a few weeks (Collip, 1934). However, a method suggested by Loeb in 1929 and later abandoned, was found useful. This classification is based on the histology of the thyroid and represents a gradation from active to inactive glands. The span of variation of the thyroid gland was divided into five classes which were numbered I, II, III, IV, V. However, no thyroid is confined to any one group but may be a member of as many as three at the same time. Stage I includes thyroids with predominantly small round follicles comprised of high epithelium and colloid of medium consistency, which usually stains a dark pink with eosin and has few absorption vacuoles. These vacuoles were interpreted by Aron in 1930 as structures associated with the process of colloid excretion (removal of stored colloid) into the blood stream. As originally suggested by Bensley in 1916 thyroid secretion is produced to a certain degree continuously and excreted into the blood stream directly. The excess colloid is stored within the follicles. Increased thyroid excretion depletes the stored colloid till only strands of colloid are seen

in the lumen. Zalesky in 1935 in the ground squirrel interprets these vacuoles mostly as artifacts. For the guinea pig they can be interpreted as signs of colloid excretion. Stage I is the predominant thyroid picture in young animals, and in adults it is almost always seen in the center of the gland. Stages II and III include successive phases of excreting and secreting thyroids. The amount of secretion in a thyroid is indicated by the height of the epithelium whereas the rate of storing and excreting colloid is indicated by the picture of the colloid. In Stages II and III the epithelium is high and quantities of absorption vacuoles are present. These two groups are merely distinguished on a quantitative basis of lesser or greater hypertrophy. Stages IV and V represent resting or colloid phases. Here dense, resting colloid is seen in large follicles with flattened epithelium. As mentioned before this might be primarily or secondarily so. In Stage IV little absorption of the stored colloid takes place and in Stage V no absorption is observed. Stage V is very similar to the picture of colloid goiter.

Uniformity of structure and physiological condition in all follicles of the same thyroid gland is very rare. An estimate of the grade of activity in a given thyroid from the histological appearance must be based on the condition of the majority of the follicles. All sections were looked over and classified and then rechecked after several months independent of the previous estimate. In the majority of cases, the classification remained consistent the second time or it varied only to a minor degree. The Tables I and II show the results of this method. Two main points were revealed in tabulating the graded thyroids: the thyroids of the gonadectomized animals showed a slightly greater variability than their controls. This is expressed by the number

of stages shown by any one thyroid at the same time. This increased variability after gonadectomy was observed in both sexes. Castrate males showed more thyroids in the resting stages than did the cryptorchid-castrates and the normal control males. Thyroids of spayed females showed the same tendency to shift towards the resting Stages IV and V.

In summarizing, therefore, one can say that the absence of the gonads for prolonged periods of time in male and female guinea pigs produces no consistent changes in the histological structure of the thyroid but a tendency to shift towards the colloid or resting stage. This means that the thyroid of the gonadectomized guinea pig is physiologically less active and tends to excrete less hormone into the blood stream and store more colloid in the follicles. This would also explain the slightly increased weight of the thyroid of the experimental animals.

C. Gonadectomized Rats

a. Males.--Nine litters of rats furnished both operates and controls for the following experiment. Ten animals were castrated at five days of age and killed at 40 to 100 days of age. Ten animals were castrated as mature adults and sacrificed after operative periods of 50 to 165 days. In each litter an equal number of animals were used as operates and controls. Throughout the entire work the heavier animals were used for operation.

The results of this experiment are shown in Table III. Gonadectomy decreased the weight of the thyroid about 16 per cent as compared to the controls. There is a probability of 1 in 20 that the difference could be due to accidents of sampling. The age of the animals at the time of the operation was not a factor in this weight decrease. Two of the nine litters, both operated

TABLE III
AVERAGE WEIGHTS FROM GONAECTOMIZED RATS
MALES

					Body Weight Grams			Thyroid Milligrams		
Number of Litter	Number of Control and Operated Each	Age in Days at Operation	Age at Autopsy	Duration of Gonad Absence in Days	Operated	Controls	Percentage Differences in Operates	Operated	Controls	Percentage Differences in Operates
21	3	5	40	35	118	106	10	13	10.9	16
55	2	5	55	50	180	120	33	19.3	20.2	- 4.4
93	2	6	90	84	277	270	2.7	21.2	35	-39
88	3	5	101	96	228	191	16	29	27.2	9.5
386	2	50	100	50	320	279	13	26.5	40	-33.7
388	2	50	100	100	236	268	-12	23.5	26	- 9.6
57	2	51	156	105	421	364	13	34.5	39	-11.5
386	2	60	160	100	305	290	4.9	26.5	36.4	-27.2
68a	2	60	225	165	261	273	- 4.4	18.5	20.5	- 9.7
Averages.....					260	240	7.7	23.5	28.3	-16.9

at 5 days of age, showed even a slight increase in the weight of the thyroid. The decrease in thyroid weight was not associated with the change in body weight. The body weight of the male castrate before and after puberty showed no significant change as compared to the controls at autopsy.

Contrary to the situation in the guinea pig castration in the rat leads to increased size of the hypophysis. The hypophyses of all the castrated animals in this group were found to be considerably heavier than those of the controls.

Histological preparations of the seminal vesicle showed that no sex hormone had been present in the castrate. Testes, seminal vesicles and prostate of all the control animals were normal.

b. Females.--Table IV summarizes the results from animals from eight litters: 24 were spayed and the same number left as controls. Four animals had been spayed before puberty whereas the twenty other animals were spayed at 50 to 90 days of age. All the females were sacrificed about 100 days after ovariectomy.

In the female rat gonadectomy produced no consistent change in the thyroid weight. Four litters showed a slight decrease in thyroid weight following ovariectomy whereas four other litters showed a slight increase in the average thyroid weight. Contrary to the situation in the male the body weight of the spayed female at autopsy was increased on the average by 23 per cent as compared with the normal controls. The weights of the hypophyses were considerably increased in the operated animals.

All control females were killed in dioestrus. The weight and structure of the uteri of the spayed females indicated that the operation on all the animals had been complete and that no ovarian hormone had been present.

TABLE IV
AVERAGE WEIGHTS FROM GONADECTOMIZED RATS
FEMALES

					Body Weight Grams			Thyroid Milligrams		
Number of Litter	Number of Control and Operated Each	Age in Days at Operation	Age at Autopsy	Duration of Gonad Absence in Days	Operated	Controls	Percentage Differences in Operates	Operated	Controls	Percentage Differences in Operates
65	4	29	129	100	298	257	14	26	28.4	- 8.4
61	2	51	156	105	311	231	26	23	19.5	15.2
72	4	58	158	100	274	204	25	20	21.7	- 7.8
386	2	60	160	100	305	194	36	26.5	23	13.5
307	5	60	160	100	252	181	28	19.3	19	1.6
451	3	58	174	116	234	188	20	18.3	18.6	- 1.5
457	2	58	174	116	254	270	33	21	21	0.2
436	2	90	200	110	231	204	12	25	31	-19.3
Averages.....					265	204	23	21.5	22.3	- 3.6

Injectons of pregnant mare serum showed no effect on the thyroid picture of the spayed and the normal female.

In the rat gonadectomy, particularly in the male, produces slight atrophy of the thyroid gland as indicated by the weight. In the female, thyroid weight after gonadectomy was variable and results were inconsistent. The body weight of the castrate male remains unchanged whereas that of the female rises significantly after gonadectomy. It is very probable that there is a correlation between this difference in body weight in the two sexes and the different effect gonadectomy produces on the thyroid weight.

c. Histology of the thyroids of gonadectomized rats.--

One thyroid fixed in Bouin's fluid was sectioned and enough sections were mounted to give a good picture of the thyroid at different levels. The sections were stained with Ehrlich haematoxylin. Some were counter-stained with Mallory-Azan.

Much of what has been said concerning the thyroid of the guinea pig applies to the situation in the rat. Compared with the guinea pig thyroid, the rat thyroid has less clearly defined follicular outlines. The stroma is more abundant. The cells of the epithelium are more irregularly arranged and the lumen is often not clearly defined. The colloid state in the rat thyroid is not so easy to analyze as that of the guinea pig. Vacuoles that are seemingly not absorption vacuoles are seen in the same follicle with dense retracted colloid. Points of consideration in examining the thyroid preparations of the rat for effects of gonadectomy were: the relative amount of connective tissue stroma to the amount of glandular epithelium, the size and shape of the acini, the height of the epithelium, the composition of the cytoplasm, the structure of the nucleus and the amount of colloid. Cells are often seen within the lumen of the follicles, some of

these are phagocytes, others are sloughed off from the epithelial lining of the lumen and others might be of still other origin.

The grading method employed in the guinea pig was not feasible in the rat because the thyroid structure was not consistent enough to warrant such a method. Contrary to the situation in the guinea pig characteristic changes could be observed in the thyroids of the gonadectomized rats. These changes were not very clearly defined nor were they seen in all the operated animals but they were consistent enough to enable one to determine fairly accurately whether a given thyroid had been removed from a gonadectomized or from a normal rat. These changes were best seen in the males, a fact which can be correlated with the more marked loss in weight in their thyroids. It made no difference how old the animal had been at the time of the operation. This is demonstrated by the fact that the two groups those operated at 5 days of age and those operated at 50 days of age, showed no difference in the animals sacrificed after 90 days of age.

The changes in the thyroids of the rats gonadectomized for at least 50 days consisted of comparatively low epithelium, associated with dense and deeply staining cytoplasm. The nuclei were often elongated, pycnotic and generally atypical. The ratio of connective tissue to that of the parenchyma was very often changed in favor of the connective tissue. This change could be expressed quantitatively with the help of planimetric measurements but measurements were not taken because other aspects of the problem seemed more important at the time.

The above mentioned changes can be interpreted as atrophy. They are seen in both males and females to a certain extent, but are very much better defined in the males. This corresponds with the observations of Anderson and Kennedy.

It seems impossible to recognize the sex of the rat by the histological picture presented by the thyroid. This excludes the possible variation of the thyroid of the female during the sexual cycle because in the presentation all control females were killed in dioestrus to simplify the problem. To my knowledge a possible correlation of changes in the thyroid with their sexual cycles has not been a subject of research in the rat. The shortness of their luteal phase makes them an unsuitable research animal for such a problem. The work of Chouke (1930) on the guinea pig has as yet not yielded very conclusive results.

So in the rat, more pronouncedly in the male, gonadectomy leads to a thyroid condition in which apparently less hormone is produced by the thyroid gland.

CHAPTER III
THE INFLUENCE OF THE THYROID GLAND ON THE
REPRODUCTIVE SYSTEM OF THE RAT

A. Introduction

In the first part of this work we have considered some of the points regarding the influence of the gonads upon the thyroid. Now the problem of thyroid-gonad relationship will have to be approached in considering the influence that the thyroid exerts upon the gonads and their functions.

Clinically the importance of the thyroid for reproduction has been known for many years. Thyroid deficiency, as well as hypothyroid conditions, are often associated with menstrual disorders in women and sterility in both sexes. Administration of dessicated thyroid is a routine procedure in cases of sterility without pathological changes.

Experimental results of thyroid removal have often been confused with disturbances resulting from removal of the parathyroids. Thyroidectomy in a mature animal decreases the basal metabolism to a greater or lesser extent depending on the species. In the immature animal thyroid removal hinders normal development and thereby retards the onset of maturity. The symptoms of thyroid deficiency can be relieved or completely overcome by feeding dried thyroid substance. Overdoses of desiccated thyroid result in increased basal metabolism and is usually accompanied by disturbances of normal reproductive function.

The question which will mainly interest us in these experiments is the degree to which the thyroid influences the gonad directly on an endocrine basis, and the degree of thyroid influence on the gonads indirectly through disturbances of general metabolism.

B. The Effect of the Thyroid on the Reproductive System of the Male Rat

a. Literature.--It seems important in view of the question of indirect or direct action of the thyroid on the gonads whether or not the thyroid acts differently in males and females. Therefore, contrary to general usage, the literature will be discussed separately for each sex.

The relationship of the thyroid to the reproductive system in the male has been the subject of comparatively little work. Particularly little attention has been paid to the effect of thyroid removal and much of the earlier work is confused with the effects of removal of the parathyroids, rendering most of the work done on dogs useless for this problem.

In the rabbit Hofmeister (1894) and Tatum (1913) observed severe injury of the testes after thyroidectomy in young and adult males. Hammett (1922, 1926) working on the rat found that normal reproduction is disturbed but attributes the changes to general metabolic disturbances. Smelser (1934) finds smaller accessories and less sperm but unaltered gonad stimulating potency of the pituitary after thyroidectomy in the male rat. The work of Dye and Morgan (1929) on the growing dog showed that thyroidectomized puppies decreased in body weight up to 68 per cent as compared to their littermate controls. But the development of the reproductive organs was in keeping with the general body development.

The hyperthyroid state produced experimentally by thyroid feeding has been studied more extensively. The main difficulty in evaluating the results is the large doses given by many investigators, producing rapid loss of weight. In general it can be said that doses low enough to permit the animals to gain weight do not effect the testes histologically. Courrier (1921) in the rat and Döderlein (1929) in the guinea pig found no hastening of sexual maturity and no effect on the structure of the testes. The behavior of the animals was influenced, however, as the males lost interest in the females in oestrus. De Costa and Carlson (1933) studied the effects of thyroid feeding on maturity. Very small doses slightly hastened development whereas larger doses slowed the onset of maturity.

The following experiments were conducted to study the effect of thyroid removal on the gonads of the adult rat as indicated by the changes in weight of the testes, seminal vesicle and pituitary. The histology of the testes, seminal vesicle and prostate were also studied. The question of the importance of the thyroid on the development of the reproductive system was studied in young male rats thyroidectomized before maturity.

b. Mature males.--To determine the effect of the thyroid on the gonad of the adult male rat 38 males from 15 litters were thyroidectomized between 43 and 330 days of age. Of each litter one-half, comprising the heavier animals, were operated and the remaining half utilized as controls.

The operation itself offered no technical difficulties. With the help of a binocular microscope an effort was made to leave the lower pair of parathyroids intact, and tetany was rare in this group of animals. When parathyroid deficiency occurred injections of a 10 per cent solution of calcium glucose was given

in daily doses from .25 cc. to .5 cc. These injections completely prevented tetany and proved to have no effect on the reproductive system as shown by three litters in which both thyroidectomized and normal males were injected.

Three litters were sacrificed from 12 to 20 days after operation, five litters between 20 and 65 days, and seven litters from 90 to 150 days after operation. Table V presents some of the data recorded from individual animals in this experiment. Table VI shows the summarized results and the percentage differences between the operated and the control animals.

A comparison of testis weights of thyroidectomized animals with those littermate controls fails to show a consistent tendency of the operation to produce changes in the size of these organs. Table VI shows that in five of the fifteen litters the testes of the operated animals were larger than those of the littermate controls, whereas in the remaining ten litters the average testis weight in the thyroidectomized males was less than that of the controls. Whatever decrease is registered is not correlated with the length of survival after thyroidectomy but in general it can be correlated with changes in body weight. With three exceptions in the fifteen litters loss or gain in testis weight of thyroidectomized animals parallels loss or gain in total body weight.

Histological study of the testes of both operated and littermate control males failed to show any definite effect upon the organ as the result of thyroid removal. Testes from each of seventy-five males reported in Table V and VI showed entirely normal organs and only one animal, No. 250, thyroidectomized at 40 days of age and sacrificed at 63 days of age possessed retarded testes. This animal definitely belongs in the immature series and will be further considered in that group.

TABLE V

INDIVIDUAL WEIGHTS FROM MALE RATS THYROIDECTOMIZED AFTER PUBERTY

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Testes Weight Grams	Seminal Vesicle Grams	Hypophysis Milligrams
250	40	63	20	106	.154	.12	6.6
26	..	63	normal control	107	2.46	.426	7.00
409	60	80	20	109	2.15	.560	..
410	..	80	normal control	179	2.98	.590	6.00
44	78	90	12	220	2.13	.880	..
36	..	90	normal control	270	3.32	.890	5.0
482	55	115	60	205	2.63	.510	10.6
485	55	115	60	180	2.72	.41	8.6
490	55	115	60	128	2.00	.28	8.2
481	..	115	normal control	200	2.51	.71	4.9
484	..	115	normal control	208	2.73	1.00	6.9
489	..	115	normal control	190	2.10	1.14	7.5
219	40	140	100	162	1.00	1.2	9.1
222	40	140	100	125	3.00	.8	7.0
224	40	140	100	126	2.3	1.06	9.0
393	..	140	normal control	160	2.65	.98	6.0
394	..	140	normal control	175	2.78	1.12	6.6
375	..	140	normal control	180	2.69	1.11	7.6
512	123	163	40	245	2.59	.54	16.0
514	123	163	40	235	2.87	.53	9.7
515	123	163	40	343	2.98	1.23	11.9
516	123	163	40	250	2.81	.51	10.8
513	..	163	normal control	244	2.89	1.09	6.9
517	..	163	normal control	299	2.91	.71	7.8
156	..	163	normal control	320	2.80	1.21	7.00
157	..	163	normal control	260	3.20	1.81	7.1
431	100	200	100	168	2.60	.87	8.00
432	100	200	100	204	3.00	1.20	14.00
433	..	200	normal control	165	2.00	.90	5.7
434	..	200	normal control	200	2.73	.84	7.8
439	110	200	90	220	2.72	1.98	8.00
446	110	200	90	223	3.00	1.24	11.00
441	110	200	90	230	2.68	1.79	14.00
445	..	200	normal control	218	2.74	1.19	7.9
446	..	200	normal control	310	3.19	1.52	10.1
126	..	200	normal control	230	2.82	.84	8.0

TABLE V--CONTINUED

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Testes Weight Grams	Seminal Vesicle Grams	Hypophysis Milligrams
442	110	200	90	250	3.20	.83	11.5
443	110	200	90	260	3.20	1.60	9.4
444	110	200	90	204	3.50	1.40	8.0
127	..	200	normal control	295	3.00	1.24	8.1
128	..	200	normal control	287	2.64	1.24	7.9
129	..	200	normal control	320	2.90	1.29	10.2
468	120	216	96	225	2.85	1.93	10.1
469	120	216	96	318	2.82	.80	12.3
474	..	216	normal control	281	3.21	.79	7.0
475	..	216	normal control	329	3.03	.82	7.0
470	120	216	96	309	3.16	1.08	12.0
471	120	216	96	200	2.95	.58	11.2
472	120	216	96	235	2.82	.73	9.0
473	120	216	96	295	3.00	.92	11.0
476	..	216	normal control	240	2.79	.88	8.2
477	..	216	normal control	250	3.16	.80	8.0
382	..	216	normal control	328	2.46	1.20	9.1
383	..	216	normal control	340	3.20	1.06	7.9
478	185	250	65	315	3.50	.82	14.0
479	185	250	65	270	2.71	1.00	12.5
480	185	250	65	220	2.11	.41	12.2
482	..	250	normal control	306	3.24	1.49	10.0
483	..	250	normal control	270	2.76	1.35	9.5
308	..	250	normal control	287	2.64	1.24	9.4
481	185	250	65	270	1.78	.21	12.0
484	185	250	65	230	2.45	.39	14.3
485	185	250	65	200	1.56	.19	13.0
381	..	250	normal control	320	2.90	1.30	..
384	..	250	normal control	300	2.84	1.10	10.0
385	..	250	normal control	250	3.40	1.36	9.8
200	150	300	150	350	3.24	1.36	..
202	150	300	150	202	2.46	.44	12.5
203	150	300	150	241	2.68	.48	13.1
201	..	300	normal control	369	2.82	1.36	9.8
246	..	300	normal control	310	3.19	1.52	10.1
401	..	300	normal control	330	2.60	1.44	10.0
213	330	365	35	362	3.44	1.24	11.2
214	330	365	35	330	3.24	1.20	11.0
215	..	365	normal control	310	3.04	1.42	9.6
153	..	365	normal control	390	3.16	1.55	10.0

TABLE VI
AVERAGE WEIGHTS FROM MALE RATS THYROIDECTOMIZED AFTER PUBERTY

Number of Litter	Number Control and Operated Each	Age in Days at Operation	Age in Days at Autopsy	Duration Thyroid Absence in Days	Body Weight Grams			Testes Grams			Seminal Vesicles Grams		
					Operated	Controls	Percentage Difference in Operates	Operated	Controls	Percentage Difference in Operates	Operated	Controls	Percentage Difference in Operates
250	1	43	63	20	106	167	-36	154	2.46	-94	.12	.43	-72
409	1	60	80	12	109	179	-39	2.15	2.98	-28	.56	.59	-5
44	1	78	90	12	220	270	-18	2.13	3.32	-36	.88	.89	-1.1
482	3	55	115	60	171	199	-14	2.45	2.43	8	.40	.95	-57
219	3	40	140	100	138	172	-20	2.10	2.70	-22	1.02	1.07	-4.6
512	3	123	163	40	268	280	-4.3	2.81	2.95	-4.7	.70	.87	-41
431	2	100	200	100	186	182	2.1	2.80	2.36	15	1.03	1.20	15
439	3	110	200	90	224	253	-11	2.80	2.82	-4.3	1.67	1.18	29
442	3	110	200	90	238	300	-21	3.30	2.85	14	1.28	1.26	1.5
468	3	120	216	96	271	305	-11	2.83	3.12	-9	.86	.81	5.8
470	4	120	216	96	260	289	-10	2.98	2.90	2.7	.83	.98	-15
478	3	185	250	65	268	288	-6.9	2.77	2.88	-3.8	.74	1.36	-45
281	3	185	250	65	233	323	-28	1.93	3.04	-36	.26	1.25	-79
200	3	150	300	150	264	336	-21	2.19	2.87	-24	.76	1.44	-47
213	2	330	365	35	346	350	-1.1	3.52	3.10	6.6	1.22	1.48	-17

In general it may be mentioned that testes of all males thyroidectomized at 60 days of age or later, and despite the length of survival after removal, showed normal spermatogenetic activity, possessed normal appearing seminiferous tubules and contained mature spermatozoa. Thirty-seven of the thirty-eight thyroidectomized males at autopsy possessed spermatozoa in the epididymis that showed motility when diluted with physiological saline solution. The interstitial tissue of operated males likewise failed to reveal any perceptible difference from that of the littermate control testis.

Modification of hormone secretion by the testis is easily determined by the state of the seminal vesicle and prostate gland as has been shown in this laboratory on numerous occasions. Whereas the prostate and seminal vesicles have been studied histologically from each of the animals of this series, weight records for seminal vesicles alone are recorded in the tables. Weights of these organs are more easily and accurately determined than are those of the prostate.

Attention to Table VI reveals that seminal vesicle weights of thyroidectomized animals were greater than those of littermate controls in four litters and less in eleven litters. All litters showing greater seminal vesicle weights were groups operated from 90 to 100 days but other similar aged groups showed seminal vesicles of operates to be lighter than the controls. As in testis weights, so in the comparison of seminal vesicle weights, there is no distinct correlation between the presence or absence of the thyroid and seminal vesicle weights.

Histological study of the seminal vesicles showed them all to be under the influence of hormone secreted by the testicle. High epithelium and the presence of secretion granules clearly

denotes the continuance of hormone secretion by the testis in the total absence of the thyroid gland. Though the microscopical condition of this organ clearly portrays the presence or absence of testis hormone, quantities of hormone above requirements for maintenance in the normal condition can only be indicated by gross weight. This criterion, as indicated above, fails to indicate clearly any consistent difference in hormone secretion by testes of controls or operated animals.

The histological state of the prostate tissue merely corroborates the results obtained by study of the seminal vesicles. All prostates of thyroidectomized animals were in a normal condition with high secretory cells containing the diagnostic light areas in acinous cells of the ventral lobe.

It is clear, therefore, that thyroid removal from mature male rats fails to induce perceptible changes in the gross weight or microscopical structure of testis, seminal vesicle or prostate glands. Germ cell formation continues in a normal fashion and hormone secretion is not perceptibly modified.

In general, the body weight of control animals exceeded that of the operated littermates to some extent. From Table V it is seen that only in one litter of the fifteen used the body weight of the thyroidectomized rats exceed that of their controls by 2 per cent. In all of the litters in this experiment the controls were heavier than experimental animals at autopsy. The percentage difference of body weights of the experimental animals and the controls at autopsy exceeded 10 per cent in eleven litters in favor of the controls.

The weight of the hypophysis of the thyroidectomized animals was increased. The completeness of the operations had been checked in some doubtful cases with Bensley's Melbola-blue method.

c. Immature males.---Three litters operated under 68 days of age and discussed in the previous chapter indicated that the effect of thyroidectomy is very much more pronounced in young animals than in adults. In order to study this developmental aspect 60 male rats were thyroidectomized before 10 days of age. Thirty-two of these animals died before they reached the age of 25 days, mainly from parathyroid tetany which set in usually between 15 to 20 days, in spite of an effort to leave two of the parathyroids in place. These can be distinguished from the thyroids in young animals with the help of a binocular microscope. Injections of calcium gluconate were found to be a satisfactory preventative against parathyroid tetany but could be continued for short lengths of time only, as continued injections produced skin infections. Administration of calcium lactate by mouth with the help of a pipette was more successful.

Of the twenty-eight animals surviving, seventeen had to be discarded because they showed regenerated thyroid tissue. Throughout the entire work animals that showed even minute traces of regenerated thyroid tissue were discarded because it was found that pieces weighing no more than a few milligrams were sufficient to supply the animal with the needed thyroid hormone.

The lack of thyroid in the young growing rat leads to severe disturbances in its general body development. But the rate of growth of the thyroidectomized animals was not noticeably different from that of their controls up to about 16 days of age. From then on their development began to lag behind normal litter-mate controls. Since bone development is not retarded by lowered metabolism to the same degree as the rest of the body, general body proportions of the rats thyroidectomized under 15 days of age were in many cases abnormal.

Eleven animals operated before 10 days of age and 10 animals from another set, operated from 10 to 26 days of age, survived beyond 25 days of age and proved to have no thyroid at autopsy. These experimental animals and an equal number of littermate controls were sacrificed at different periods from twenty-five to eighty days of age. Duration of the thyroidless condition ranged from 12 to 66 days.

Table VII gives an account of the results obtained at autopsy from these males. Table VIII summarizes the results according to litter average and give the percentage changes of the body weight, testes and seminal vesicles.

It will be seen that throughout the entire series of male rats thyroidectomized before puberty, a correlation can be noted between the decrease of body weight and the time at which thyroidectomy was performed. In two litters autopsied at 23 and 24 days of age the body weight decrease of the experimental animals was 22 and 23 per cent. There was a 50 per cent increase in the six litters that had been thyroidectomized under 10 days of age and killed from 30 to 38 days of age. On the whole, one can say that the younger the animal at the time of operation the greater is the retarding influence of thyroidectomy on general body growth.

Table VIII shows that parallel to the decrease in general body weight went a decrease in testis weight. This percentage was generally larger than the percentage decrease of the body weight. It must be kept in mind that general body development and development of the testis as expressed in weight do not run parallel; rise in testis weight is far more abrupt between the ages of about 25 to 40 days than the increase in body weight.

TABLE VII

INDIVIDUAL WEIGHTS OF MALE RATS THYROIDECTOMIZED BEFORE PUBERTY

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Testes Weight Grams	Seminal Vesicle Grams	Thyroid Milligrams
254	8	23	15	24	.128	10.0	..
236	..	23	normal control	31	.250	11.2	3.8
253	8	24	16	27	.142	8.0	..
237	..	24	normal control	35	.292	10.0	4.2
248	14	28	14	32	.203	5.4	..
250	..	28	normal control	36	.300	6.6	3.1
326	10	30	20	30	.158	9.1	..
377	..	30	normal control	70	.808	24.2	18.0
280	8	31	23	41.5	.232	8.4	..
283	..	31	normal control	62	.560	8.2	7.1
250	10	35	25	40	.292	2.6	..
377	..	35	normal control	71	.812	24.0	12.0
376	10	36	26	36	.162	3.0	..
260	..	36	normal control	90	1.010	24.6	7.1
256	10	36	26	44	.272	10.6	..
257	10	36	26	35	.102	7.6	..
258	10	36	26	39	.212	17.0	..
267	..	36	normal control	84	1.80	26.2	6.1
466	..	36	normal control	90	1.69	23.0	12.1
467	..	36	normal control	88	1.32	25.0	12.0
238	10	38	28	41	.200	9.7	..
239	10	38	28	37	.128	10.1	..
340	..	38	normal control	100	1.57	31.0	9.2
341	..	38	normal control	95	1.40	27.0	8.4
244	26	38	12	49	.420	8.8	..
245	26	38	12	48	.500	10.8	..
246	26	38	12	46	.440	8.0	..
247	..	38	normal control	65	.620	10.2	4.8
230	..	38	normal control	106	1.360	56.0	9.5
334	..	38	normal control	102	1.68	80.0	6.6

TABLE VII--CONTINUED

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Testes Weight Grams	Seminal Vesicle Grams	Thyroid Milligrams
240	15	48	33	76	.860	19.0	..
242	..	48	normal control	100	1.66	27.0	6.3
327	17	52	35	106	1.60	58.0	..
328	..	52	normal control	137	2.14	117.0	10.0
516	20	56	36	105	1.81	190.0	..
518	..	56	normal control	156	2.44	230.0	27.9
361	12	60	48	64	.68	22	..
362	..	60	normal control	135	2.76	180	25.0
414	20	60	40	106	1.45	224	..
413	..	60	normal control	150	2.46	670	15.0
470	28	70	42 (regen)	160	2.28	420	..
473	..	70	normal control	170	2.20	510	16.0
416	14	80	66	64	.68	220	..
417	..	80	normal control	135	1.92	480	21.0

TABLE VIII
AVERAGE WEIGHTS OF MALE RATS THYROIDECTOMIZED BEFORE PUBERTY

Number of Litter	Body Weight Grams				Testes Grams			Seminal Vesicle Milligrams			
	Number Control and Operated Each	Age in Days at Operation	Age in Days at Autopsy	Duration Thyroid Absence in Days	Operated	Controls	Percentage Differ- ence in Operates				
254	1	8	23	15	24	.128	.250	-48	10	11.2	-10.7
253	1	8	24	16	27	.142	.292	-51	8	10.1	-20
248	1	17	28	14	32	.203	.300	-32	5.4	6.6	-18
326	1	10	30	20	30	.158	.808	-80	9.1	24.2	-61
256	1	8	31	23	41.5	.232	.560	-58	8.4	8.2	-89
376	1	10	35	25	40	.280	.812	-64	2.6	23.6	-65
256	1	10	36	26	36	.162	1.01	-84	8	24.6	-54
238	2	10	38	26	39.3	.162	1.63	-90	11.4	24.7	-66
244	1	26	38	12	49	.42	1.49	-89	9.9	29	-
245	1	26	38	12	61	.45	.62	-	8.8	10.2	-81
240	1	26	48	33	91	.86	1.08	-51	9.2	48.7	-81
327	1	15	52	35	76	1.60	1.66	-48	19	27.6	-31
516	1	20	56	36	106	1.81	2.14	-25	58	117	-50
561	1	20	60	48	105	1.81	2.44	-26	190	230	-17
414	1	12	60	48	64	.68	1.76	-61	22	180	-88
414	1	20	60	40	106	1.45	2.46	-49	224	670	-66
416	1	14	80	66	64	.68	1.92	-64	220	480	-54

In three litters killed before 28 days of age, the weight of the testes decreased about 40 per cent. The histological picture of these organs were not very different from those of the controls. Normal looking tubules, showing many mitoses, were present. The amount of interstitial tissue was not materially changed and the whole picture of the testes of the operated animal appeared retarded; they were similar to testes of animals four to five days younger than the controls.

The weight of seminal vesicles of these animals was almost the same in experimental and control animals and similar in histological structure. They possessed secretory epithelium but no secretion granules. The prostates of the operates in these three litters did not differ markedly from those of the controls. They showed the typical light areas of the secretory state in the acinous cells.

Six litters killed from 30 to 38 days of age showed a more pronounced decrease in testis weight, namely 58 to 90 per cent. At this age, sperm heads, i.e., spermatozoa in the final stage of development just before they form a tail, appear in the controls. The appearance of sperm heads has been used by Moore (1936) as a dependable indicator of the state of development of spermatogenetic activity. None of the operated animals showed sperm heads before 48 days of age. But the testes of the thyroidectomized animal at 30 to 40 days of age, though retarded in development failed to show pathological states but corresponded to animals of a younger age. Most of the operates sacrificed at 30 to 38 days of age and operated before 10 days of age had testes corresponding to those of rats of about 25 days of age. An exception to this observation was found in the case of two animals which showed pathological changes. These changes consisted of a

very low layer of germinative epithelium within the seminiferous tubules and a correspondingly larger lumen. Normally the lumen in the seminiferous tubule in the controls of 25 days of age and the thyroidectomized animal of about 35 days of age was very small.

The seminal vesicles in this group of operated animals sacrificed at 36 days of age were decreased about 55 per cent. This is a smaller decrease than could be expected from the corresponding testes weights. Their histology, like those of the testes, was similar to that in normal animals of a younger age. All showed low epithelium without secretion granules but the lumen of the gland was filled with a secretion-like mass. The seminal vesicles, therefore, did not correspond to the age of the animal but rather to its body development at the time of autopsy.

The prostates in these animals were less developed in the experimental animals than in the controls. They did show light areas in the acinous cells but the height of this secretory epithelium was less than normal and the amount of secretion was decreased.

The time of operation determines the severity of the effect that loss of thyroid produces on the development of the reproductive organs. This was borne out by two litters which had been thyroidectomized at 26 days of age and sacrificed at 38 days of age similar to those animals discussed above. Their body weight was decreased only about 25 per cent. The testes of these experimental animals were considerably more advanced in development than those from animals of the same age but operated at 10 days of age. However, sperm heads were not present in the tubules. Seminal vesicle and prostate in the operates at 38 days of age were histologically indistinguishable from those of their controls

and were far more developed than the spermatogenetic state would lead one to expect. Only their weight was decreased.

A litter operated at 15 days of age showed at 48 days of age the first sperm heads observed in any of the operated animals. Secretion granules first appeared in the seminal vesicles in an animal 56 days of age and operated at 20 days; in normal males these appear at approximately day 36.

On the whole one may say that spermatogenetic activity as well as hormone secretion was retarded by thyroidectomy. This retardation was correlated with retardation in the general body development. The time at which thyroidectomy was performed is expressed in the amount of developmental retardation. The development of the gametogenetic tissue in the testis indicates this correlation best. The development of the hormone-secreting capacity of the testis, as expressed in the histology of the seminal vesicle, was also correlated with the general body development of the animal. At this age the prostate, however, is a dependable indicator of testis hormone because it has been demonstrated that to a certain degree the development is independent of the presence of the gonads (Price, 1934). Light areas would be present even in castrate animals up to 35 days of age. The slight indication that the prostate is sometimes more advanced in development than the corresponding testis would warrant could be explained on this basis.

In all these prepuberally operated animals the weight of the hypophysis was about the same as in the controls, thereby considerably increasing the proportionate weight of the hypophysis of the operates. The same phenomenon was found for the adrenals. Increase in the weight of these organs up to 33 per cent was found in animals operated under 10 days of age.

d. Thyroid fed males.--Ten adult male rats were fed Armour's dessicated thyroid. Six littermates were used as controls. The method employed to administer fairly accurately a certain dose was subject to many preliminary trials. Feeding thyroid powder in small capsules was found unsatisfactory. Mixing thyroid with the animals' food and calculating the amount consumed was found to be inaccurate because the rats habitually throw much food around in the cage. Finally a suspension of thyroid in a given amount of water was prepared and administered through a graded pipette. Most rats will take thyroid willingly by this method after a few days.

Two animals received 0.1 gr., 2 received 0.2 gr., 2 received 0.3 gr., and 4 animals received 0.4 gr. daily for a period of 15 days. Within this range the body weight of all the experimental animals dropped but none of the animals died.

Motile sperm were found in the epididymis of all the thyroid fed animals. The weights of the testes of the experimental animals were normal. Their histology revealed nothing abnormal. The secual accessory glands, the seminal vesicles and the prostate, showed no change from the normal, either in weight or in histology.

The conclusion can be drawn that the amount of time that these rats were fed thyroid was not sufficient to produce any effects on either the spermatogenic or the hormonal activity of the testes.

C. The Effect of the Thyroid on the Reproductive
System of the Female Rat

a. Literature.--Far more work has been done on the effect of the thyroid on reproduction in the female than in the male. The female rat is a particularly suitable animal for the study of the effect of the thyroid on the sexual cycle as the vaginal smear method enables the experimenter to follow the oestrus cycle with ease.

The effect of thyroid removal has been studied in the female rat by Hammett (1922, 1926) who found that the experimental animals matured more slowly, producing less young with a high mortality rate. In the rabbit Hofmeister (1894), Tatum (1913) and Kunde, Carlson and Proud (1929) found severe degeneration of the ovary following thyroidectomy.

Evans and Long (1921) followed the length of the oestrus cycle in thyroidectomized rats and found no change from normal. Their work was followed by the experiments of Lee (1925) and Richter (1933) who quite definitely showed that the cycles of the thyroidectomized females were longer and irregular.

The fertility of the thyroidectomized female depends greatly on the species. The work of Chandler (1930) on rats shows that the number of young conceived and the sex ratio of the embryos is unchanged but that the lack of thyroid greatly reduces the number of young born. Marza on the guinea-pig (1929) finds that the young born are smaller at birth and slower in development. Thyroidectomy in human patients with hyperthyroid conditions does not result in abortion. Fahrni (1930) records 85 cases of normal delivery after thyroidectomy but does not mention whether thyroid substance was given.

Feeding desiccated thyroid has been done extensively in the laboratory rodents. Cameron and Aimes (1926), in mice and guinea pigs, Weichert and Boyed (1930) in rats, have noticed that light doses will lengthen the cornified period in the cycle whereas heavy doses stop the cycle completely. Hoskins (1910), Pighini (1925) and Seidentopf (1930) in the guinea pig, Kunde Carlson and Proud (1929) in the rabbit and Weichert (1930) in the rat all found that desiccated thyroid fed to pregnant animals, even in small quantities, led to abortion or reabsorption of the embryos. Finally the problem was attacked from another angle by Reiss and Pereny (1928) and confirmed by Van Horn (1931) that injection of thyroid into castrate rats raised the threshold of response to oestrin. This has been interpreted by the latter as a result of oestrin excretion by the heightened metabolism.

The following experiments were devoted to a study of the effect of thyroidectomy in mature females as indicated by the weight and histological changes in the ovary and uterus, the effect on the oestrous cycle, on fertility and the number of young born. Immature females were thyroidectomized in order to see the importance of the thyroid for the development of the reproductive system.

b. Mature females.---In the female rat the effect of thyroidectomy on reproduction can be detected in the living animal by following the oestrous cycle with Long and Evans smear technique. In the majority of the animals used in this experiment, sexual cycles had been established before thyroidectomy and were studied for the remainder of their lives. After the cycle of an animal was once established smears were taken but once daily to avoid irritation of vagina and disturbance of the cycle.

Forty-three female rats from 19 litters were operated at ages ranging from 60 to 330 days and killed from 80 to 450 days of age. An equal number of normal littermates served as controls. The operation was checked in doubtful cases by perfusion with Melbala's blue into the carotid artery (a method suggested by Dr. Robert R. Bensley).

Six litters were sacrificed after thyroidectomy for periods under 100 days and thirteen litters after periods from 100 to 200 days. Tables IX and X show that the weight of the ovaries were practically the same in experimental and control animals. The difference in individual litters between operates and controls was definitely within the range of normal variability of ovarian weight. The histology of the ovaries, however, revealed that in many operated animals thyroidectomy was followed by a scarcity of corpora lutea. The number of the corpora lutea as well as their size and the amount of luteinization was decreased. This was seen in the majority but not in all the operated females. To facilitate comparison all animals were killed in dioestrus, usually when all cornified cells had disappeared from the vaginal contents and mainly leucocytes were present. In ovaries of experimental animals many degenerate follicles were seen. Also a number of structures appeared to be only partly luteinized and partly degenerated. These histological changes in the ovaries of experimental animals, indicating a decrease in the amount of corpora lutea, were not always consistent. This phase deserves further study. Exact timing of death in relation to the cycle and development of quantitative methods for estimating the amount of corpora lutea present will be essential. However, my study makes it seem probable that thyroidectomy interferes with luteinization.

TABLE IX
INDIVIDUAL WEIGHTS FROM FEMALE RATS THYROIDECTOMIZED
AFTER PUBERTY

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Ovary Weight Grams	Uterus Weight Grams	Hypophysis Milligrams	Thyroid Milligrams
412	60	80	20	124	50	.72	7	..
411	..	80	normal control	130	68	.90	11	28
48	76	90	14	197	78	.59	8	..
47	..	90	normal control	202	81	.68	7.5	22
186	64	138	74	128	62	.42	16.1	..
187	64	138	74	90	30	.15	9.1	..
185	..	138	normal control	159	68	.68	10.8	16.2
189	..	138	normal control	179	94	1.08	12.2	18.9
189a	64	138	74	116	70	.22	14	..
190	64	138	74	81	35	.12	11.4	..
191	64	138	74	170	82	.57	14.6	..
192	64	138	74	85	37	.14	9.8	..
130	..	138	normal control	219	48	.49	10	26
131	..	138	normal control	230	24	.35	..	25
132	..	138	normal control	221	32	.40	9.2	20
133	..	138	normal control	219	25	.36	8.7	25
418	100	200	100	200	56	.50	12.5	..
419	100	200	100	158	71	.22	10	..
420	100	200	100	175	78	.39	15	..
424	..	200	normal control	188	130	.25	7	19
225	..	200	normal control	165	86	.48	6.3	20
226	..	200	normal control	168	82	.51	8	17.9
422	100	200	100	213	122	.42	11.2	..
223	100	200	100	138	58	.20	12	..
227	..	200	normal control	190	125	.38	9.8	21
148	..	200	normal control	298	29	.84	8.7	32

TABLE IX--CONTINUED

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Ovary Weight Grams	Uterus Weight Grams	Hypophysis Milligrams	Thyroid Milligrams
435	100	200	100	186	58	.28	13	..
149	..	200	normal control	222	85	.70	10	18
403	100	200	100	240	65	1.15	18	..
437	..	200	normal control	220	133	.70	9.2	34
487	140	205	65	142	50	.25	10.6	..
486	..	205	normal control	188	62	.38	8	25
231	58	227	169	159	78	.61	16	..
232	58	227	169	145	38	.26	11.8	..
233	58	227	169	181	86	.65	20	..
234	..	227	normal control	218	73	.43	14.4	24
235	..	227	normal control	198	68	.59	12.8	25
130	..	227	normal control	185	27	.16	13	16
229	85	235	150	155	28	.71	12.2	..
230	85	235	150	179	86	.45	15.9	..
226	..	235	normal control	140	81	.75	10	15.7
227	..	235	normal control	184	104	.86	8.9	16.5
269	74	236	160	171	72	.45	8.8	..
271	74	236	160	212	65	.46	16.1	..
274	74	236	160	191	42	.54	17.6	..
270	..	236	normal control	183	86	.60	16	23.3
273	..	236	normal control	202	76	.50	12.2	18.2
151	..	236	normal control	241	24	.89	12.5	25
447	130	250	120	240	104	.30	10	..
448	130	250	120	230	121	.41	18	..
481	..	250	normal control	279	140	.61	8.2	27
482	..	250	normal control	192	50	.15	7.4	24

TABLE IX--CONTINUED

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Ovary Weight Grams	Uterus Weight Grams	Hypophysis Milligrams	Thyroid Milligrams
216	330	365	35	281	50	.40	14.5	..
217	330	365	35	208	14	.82	14.5	..
218	..	365	normal control	190	14	.73	9.1	14.1
143	..	365	normal control	200	47	.60	10	24
340	150	350	200	201	86	.51	14	..
341	150	350	200	180	58	.28	14	..
342	150	350	200	190	78	.72	15	..
343	150	350	200	192	106	.47	16	..
69	..	350	normal control	226	87	.50	9.8	23.5
70	..	350	normal control	267	61	.68	11	33.4
71	..	350	normal control	274	108	.71	10.1	30.1
72	..	330	normal control	264	91	.87	9.8	21.9
161	330	480	150	254	116	.50	16.6	..
162	330	480	150	235	102	.64	12.4	..
163	330	480	150	150	82	.36	13.9	..
164	330	480	150	245	132	.85	13	..
165	330	480	150	210	49	.55	10	..
167	330	480	150	235	164	.80	15	..
168	330	480	150	220	108	.41	8.6	..
179	300	450	150	256	146	.66	16.5	..
180	300	450	150	195	80	.53	13.2	..
182	300	450	150	221	53	.40	14	..
183	300	450	150	226	156	.74	16	..

Uterine weights were decreased in the experimental animals more than would have been indicated by their corresponding ovarian weights. This applies to average weights for many animals since individual variability is great. The histological structure of the uteri of the thyroidectomized animals was normal. Thyroidectomy apparently affects the amount of ovarian hormone but the threshold necessary for maintenance of the normal histological structure of the uterus was not crossed.

The decrease in body weight in the thyroidectomized females was statistically significant. As Table X shows in three litters out of nineteen no change in body weight was observed in the experimental animals. In four litters the comparative weight decrease was below 10 per cent; in four, from 10 to 20 per cent; in three from 20 to 30 per cent and in four above 30 per cent.

c. Immature females.--Fifty-four female rats were thyroidectomized before maturity. Of those, thirty animals in seventeen litters, operated at 44 days of age, survived and proved at autopsy to have been completely thyroidectomized. The same difficulty as described for the males was encountered in rearing these animals. Sixteen animals could be raised beyond sexual maturity.

The individual weights of organs obtained at autopsy from the experimental animals and controls are recorded in Table XI. The percentage of difference in weight and the litter averages are to be seen in Table XII.

The decrease in body weight of the female thyroidectomized before puberty is within the same range as described more in detail for the immature thyroidectomized male. The amount of change depends upon the time at which the operation was performed and the duration of the thyroidless state. Operated females throughout

TABLE XI

INDIVIDUAL WEIGHTS FROM FEMALE RATS THYROIDECTOMIZED
BEFORE PUBERTY

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Ovary Weight Grams	Uterus Weight Grams	Hypophysis Milligrams	Thyroid Milligrams
305	10	30	20	29	24	.035	4	..
306	..	30	normal control	59	38	.032	4	9.4
324	8	30	22	9	8.6	.011	3.7	..
325	..	30	normal control	29	20	.022	2.8	2.8
287	8	31	23	19	14.6	.023	3.8	..
288	..	31	normal control	55	37	.053	6	9
401	10	33	23	15	6	.020	3	..
402	10	33	23	22	6	.029	2.2	..
403	10	33	23	25	10	.026	3.7	..
404	10	33	23	27	12	.023	3.5	..
405	..	33	normal control	52	26	.031	2.1	7.2
307	..	33	normal control	53	46	.024	3.8	8.2
308	..	33	normal control	51	41	.029	4	8.4
250a	..	33	normal control	55	25	.030	3.5	8.2
258a	30	40	10	38	5.3	.024	2.3	..
259	30	40	10	41	6.9	..	2	..
250	..	40	normal control	55	8.2	..	1.8	3.5
251a	..	40	normal control	42	7.3	.031	2	3
461	25	50	25	38	14	.022	2.2	..
462	25	50	25	49	11.8	.030	2.7	..
463	25	50	25	38	12	.042	1.9	..
464	25	50	25	64	25	.095	3.4	..
465	..	50	normal control	87	35	.059	2.8	10
465a	..	50	normal control	90	132	.040	3.8	13
466	..	50	normal control	88	38	.046	4	12
467	..	50	normal control	69	22	.012	3	11.7
290	22	57	35	90	51	.074	7.9	..
292	..	57	normal control	120	68	.154	2.8	13
412	13	63	50	60	23	.040	5	..
413	..	63	normal control	125	66	.400	3	16

TABLE XI--CONTINUED

Animal Number	Age in Days at Operation	Age in Days at Autopsy	Duration of Thyroid Absence in Days	Body Weight Grams	Ovary Weight Grams	Uterus Weight Grams	Hypophysis Milligrams	Thyroid Milligrams
421	14	80	66	68	26	.040	5.9	..
422	14	80	66	67	22	.049	5.8	..
423	..	80	normal control	118	83	.590	8	21
428	..	80	normal control	130	85	.480	6.2	24
251a	10	90	80	60	14	.046	6	..
408	..	90	normal control	130	85	.250	8	17
330	17	110	93	115	65	.580	12	..
365	..	110	normal control	138	75	.870	6.9	12
301	17	115	98	78	30	.860	8	..
291	..	115	normal control	149	85	.270	8.6	18
364	44	134	90	140	82	.530	8.8	..
365	..	134	normal control	158	85	.680	8	16.5
251	16	140	124	125	63	.360	12	..
253	16	140	124	35	16	.032	2	..
252	16	140	124	72	28	.102	7.6	..
254	..	140	normal control	141	79	.282	5.8	26
502	..	140	normal control	147	54	.100	9.2	22
501	..	140	normal control	150	75	.400	7.1	28
252a	14	134	120	71	33	.035	5.6	..
254a	14	134	120	88	19	.029	5.4	..
255a	14	134	120	89	21	.033	5.3	..
253a	..	134	normal control	150	71	.380	6.1	21
256a	..	134	normal control	140	78	.410	8	22
257a	..	134	normal control	160	84	.670	7.5	19

TABLE XII

AVERAGE WEIGHTS OF FEMALE RATS THYROIDECTOMIZED BEFORE PUBERTY

Number of Litter	Number Control and Operated Each	Age in Days at Operation	Age in Days at Autopsy	Duration Thyroid Absence in Days	Body Weight Grams			Ovaries Milligrams			Uterus Milligrams		
					Operated	Controls	Percentage Difference in Operates	Operated	Controls	Percentage Difference in Operates	Operated	Controls	Percentage Difference in Operates
305	1	10	30	20	29	59	-51	24	38	-36	35	32	8.5
324	1	8	30	22	9	29	-70	8.6	20	-57	10.6	21.9	-51
287	1	8	31	23	19.7	54.9	-65	14.6	37	-60	23	53	-56
401	4	10	33	10	22	52.5	-58	8.5	34.5	-76	24.5	29	-15
458	3	30	40	10	39	48	-19	6.1	7.8	-22	..	..	..
461	4	25	50	25	47	83.5	-44	14.2	56.7	-75	47.2	37	21
290	1	22	57	35	90	120	-25	51	68	-25	74	154	-52
412	1	13	63	50	60	125	-52	23	66	-65	40	400	-90
421	2	14	80	66	68	124	-45	24	84	-71	44.5	53	-16
251	1	10	90	80	60	130	-54	14	85	-83	46	250	-82
330	1	17	110	93	115	138	-17	65	75	-133	580	870	-33
301	1	22	110	93	78	149	-47	30	85	-65	86	270	-68
364	4	44	134	90	90	140	-11	82	85	-3.5	530	680	-22
251	1	16	140	124	77.5	147	-47	35.8	66	-45	164	261	-37
252	3	14	134	120	82	150	-45	24.3	79	-70	32.3	486	-93

life were consistently about 40 per cent lighter in weight than their littermate controls.

The ovaries of the experimental animals showed a weight decrease corresponding roughly to decrease in body weight. Little significance can be attached to this, however, as little or no correlation exists between body weight and ovary weight in normal animals. The histological picture of the ovary of the thyroidectomized female did not differ fundamentally from that of the control. To a certain degree the same phenomenon as observed in the males held here. The size of the ovary does not correspond to the age of the animal but to the developmental state of the body. A scarcity of corpora lutea was noted in those animals that were killed after ovulation had occurred. The situation was very much like that discussed for adult females.

The uterine weights were too variable to allow definite conclusions to be drawn but on the whole there is a tendency for better uterine development than the state of the ovaries would warrant.

Adrenals and hypophysis were relatively much larger in thyroidectomized animals. The weight differences were more pronounced in those animals operated at earlier ages.

d. Sexual cycles in thyroidectomized females.--The ovarian cycle in the rat is expressed in changes in the cell lining of the vagina and can, therefore, be easily observed. The cycle has been followed in all the animals before thyroidectomy and only animals with regular cycles were employed in these experiments. The autopsy findings of the experimental females have been given above and only those animals with no thyroid regeneration were considered. This was rigorously adhered to because of the possibility that even very small regenerated areas would be sufficient to maintain the cycle at its normal rate.

The normal adult female has an oestrous period every 4 or 5 days. Directly after the operation in most thyroidectomized individuals the cycle would cease for a period of from 6 to 10 days. This might be due to operative shock and need not be a specific action of thyroidectomy.

In females thyroidectomized after maturity the first cycle was very irregular but usually the second cycle was again typical in length for the new rhythm to be established after the operation. The cycles of the experimental animals ranged from 5 to 18 days. The cycles of the thyroidectomized females, though prolonged, were fairly constant with the exception of those irregularities mentioned on the following page. Regular smears were recorded for 37 females that proved to be completely thyroidectomized and of 32 littermate controls. In addition vaginal smear records were obtained for 40 thyroidectomized females and their controls in an experiment concerning the fertility of the thyroidless rat. The youngest animals in this series at the time of operation were 58 days of age and the oldest animals operated were 330 days of age. It seems pretty definite that the age of the animal is not a factor influencing the length of the cycle. Smears were continued from 65 days to 220 days in a group of animals.

The two main points to be considered in changes in the cycle are first the length of time between two oestrus peaks before and after operation, and second the relative length of the different parts of the cycle. All animals had longer cycles after the operation than before. The average in the experimental animal was between 7 and 8 days as compared with 4 and 5 days in the control animals. In a few animals the cycle was only very slightly lengthened while in others some periods were even shorter than the controls, but the cycle average of every experimental

animal was longer after the operation than before. The control animals whose smears were taken guarded against error due to fluctuations on account of other reasons than the operation.

The relative change of the different phases within the cycle was not so regular. In most cases the length of the dioestrus period was lengthened, the vaginal fluid containing many leucocytes. Another quite characteristic change was the long persistence of cornified cells; in other words, leucocytes did not appear directly after the oestrus stage. I am rather inclined to believe that it is not a longer period of true oestrus, because the female will not willingly accept the male at this period; rather it is a delay in the infiltration of leucocytes.

It needs to be emphasized that the oestrous cycle of the thyroidectomized female rat loses the strict regularity of the normal animal. A frequent, and apparently significant cycle type was characterized by a dioestrous interval of from 10 to 20 days followed by a shorter (3 to 5 days) dioestrous interval, and again by a long dioestrous interval. This was observed frequently enough to perhaps attach some significance to the phenomena.

Very small thyroid remnants were sufficient to maintain the length of the cycle at the original level. At the beginning of this work in the preliminary studies many females retained their characteristic cycle length after the operation or after a few weeks returned to their short initial cycle length. In almost every case regenerated thyroid tissue was found. Two animals with normal cycles and in which no thyroid tissue could be detected, suggest the possibility that in some animals thyroid tissue might be present in the thymus or in some other region distant enough from the normal thyroid loci to escape observation after sectioning.

The idea that very small quantities of thyroid are sufficient to maintain the cycle is substantiated by an experiment in which six thyroidectomized females were fed thyroid. These animals whose cycle after the operation had been regular 8 day cycles were fed dessicated thyroid powder by mouth with the help of a pipette. Two animals received 0.06 gr. every day, two received 0.03 gr. and the remaining two received 0.1 gr. daily. The two animals receiving 0.06 gr. had 11 and 12 oestrous periods, respectively, from the 28th of January to the 10th of March which gives an average cycle of 3.8 and 3.5 days. This was a shorter cycle for both animals than those before the operation. The other four animals in this group responded less regularly in the same direction. However, in all six animals treated, the cycle was considerably shortened. Thyroid feeding in larger doses causes the cycle to stop just as in normal animals.

Thyroidectomy before opening of the vagina causes a delay in the onset of maturity. Sixteen animals operated before 44 days of age could be raised beyond the onset of sexual maturity. The average vaginal opening occurred on day 62 (50 to 111 day) in comparison with day 51 for littermate controls.

Cycles of females thyroidectomized before puberty, similar to those operated as mature animals, are very irregular. The intervals between oestrous periods in animals directly after maturity are longer but the same applies also to normal animals.

One interesting observation was characteristic for all animals operated before 20 days of age. Every thyroidectomized animal will grow, gain weight and reach sexual maturity though slowly, as indicated by the onset of the oestrous cycle (not by the opening of the vagina). Usually from 5 to 10 weeks after puberty, the animal begins to lose weight, cycles cease and in

some animals the vagina became closed. All females operated before maturity showed this phenomena to a more or less pronounced degree. The animals all died before 135 days of age, giving the picture of increasing weakness. This phenomenon, so like the picture of senescence, might be due to the continued lack of thyroid during development. The parathyroid may have some influence in the sequence of events but it seems unlikely, since after about 40 days of age parathyroid difficulties, such as tetany, are rare in the strain of rats used. To make sure that the calcium intake had no influence on the cycle both experimental animals and controls were given about $\frac{1}{2}$ per cent calcium lactate per total food weight. This seemed to be sufficient to maintain the animal at a normal calcium level and not disturb the normal cycles because, with one exception, the cycles of the littermate controls, receiving calcium were within normal range.

e. Fertility of thyroidectomized females.--To test the fertility of the thyroidectomized female rat 40 adult animals were deprived of their thyroids. Animals operated before maturity were unsuitable for this experiment because they never reached the body weight of the normal controls and their stunted general development would have presented an added complication. The animals used consisted of one group of 15 females that had been bred previous to thyroidectomy and raised normal litters, and a second group of 25 virgin females, most of them about 100 days of age.

The previous work on the cycle of the thyroidectomized female had demonstrated quite clearly that the effect of thyroidectomy on the cycle is permanent. The length of time elapsing after operation does not influence the effect of thyroidectomy on the length of the cycle, excluding those cases in which regeneration occurred. In addition to the evidence obtained by taking

vaginal smears, the animals were placed with breeding males at varying intervals after operation to see whether or not their fertility would be changed by the length of the thyroidless condition. Twenty females were transferred to breeding cages directly after they had fully recovered from the operation, usually about two or three days. The remaining twenty females were bred at four weeks, eight weeks and six months after the operation.

Smears were obtained from all animals in the breeding cages to determine the time at which fertilization occurred. This naturally varied according to the length of time that elapsed between the operation and the first occurrence of oestrus thereafter. It also varied with the length of sexual cycle that was established after thyroidectomy, because many of the animals were not impregnated at their first oestrous period.

Since there were an insufficient number of littermates to furnish adequate controls for all the experimental animals, a comparable number of normal animals were selected from the stock to provide additional data. The number of young per litter was also compared with the averages obtained from breeding records.

As soon as the smear indicated that fertilization had occurred, the pregnant female was transferred to a single cage. Preliminary experiments had shown the necessity for some calcium substitution therapy in pregnant females; therefore, these experimental females received calcium lactate daily with their bread and milk mixture. Parathyroid tetany occurred in but one animal so treated and this one finally died. It is not quite clear to what extent parathyroid insufficiency may exist without expressing itself in tetany and what effect it might have on reproduction. In the dog, puppies have been successfully raised from parathyroidectomized mothers that were fed calcium in various forms and

the dog is notably more sensitive to parathyroid deficiency than the rat.

Impregnation seemed to be little influenced by the duration of the thyroidless state. Virgin females and animals that had previously littered, bred at once, at four weeks, eight weeks or six months after the operation. The normal females were almost always impregnated before the experimental animals but this is probably influenced to some extent by operative shock. The results of this experiment are summarized in Table XIII.

Eleven animals failed to conceive after they had been with the males for more than eight weeks. Two of these had very irregular cycles and very possibly had irregular cycles previous to the operation. Nine animals with regular, though not normal, cycles failed to conceive; these consisted of approximately equal numbers in the two groups. Eight animals died during pregnancy; one before the twenty-first day of pregnancy and all others at the twenty-second, twenty-third or twenty-fourth day of pregnancy, as calculated from the smear record. This method is not as accurate as observing copulations but is sufficient for the present purpose. Embryos, fully developed, were found in the uteri of these animals, dead before parturition, and in two females partial embryonic resorption was noted. Later on animals that had failed to litter by the twenty-second day of pregnancy were injected with pituitrin. This method was employed successfully in four cases, one of which died after delivering two live and eleven dead young. Six animals absorbed their young around the fifteenth day of pregnancy. Mothers were sacrificed to count the number of embryos in cases where palpation indicated resorption. Finally, seven females littered normally. Some of the young were born alive in each of these litters. One female delivered four living, and

TABLE XIII
FERTILITY OF THYROIDECTOMIZED FEMALE RATS

Number of Mothers	Fate of Mothers	Offspring		
		Live Young	Dead Young	Resorbed
11	Never impregnated	..	..	..
8	Died in late pregnancy	..	52	6
1	Died in tetany	..	6	..
4	Never littered	..	..	48
4	Alive (pituitrin)	4	43	..
1	Alive (normal)	20	18	5
Total..31		24	119	59

three dead young and retained five almost absorbed young in the uterus.

All experimental animals were sacrificed to ascertain the completeness of the operation. Three females that had had normal litters were discarded on account of thyroid regeneration. Raising the live offspring from the experimental animals proved difficult because they were extremely frail and several mothers failed to lactate sufficiently. Young were given to foster mothers in such cases. The total number of embryos born alive, dead or absorbed during pregnancy, averaged 7.8 per litter. This is near the normal average in our colony, considering the twenty-five virgin females.

Thyroidectomized males mentioned earlier in this paper sired one litter each from thyroidectomized females. One litter contained 9 young at birth, two of which finally reached puberty. The other litter contained 7 young, 6 of which could be raised. These young developed considerably slower than the normal rate. At 70 days of age the four males in those two litters averaged 90 grams body weight as compared to an average of 200 grams for the males of a litter of the mother's control sister of the same age. The females in those same litters weighed 75 grams average body weight as compared to 125 grams in the controls at 70 days of age. In spite of their slower growth, these animals finally reached sexual maturity and were successfully bred. This phase of the work was discontinued as it was not within the scope of this investigation but the question of the development of animals bred from thyroidectomized parents is of enough interest to be studied in more detail.

CHAPTER IV

DISCUSSION OF RESULTS

An attempt has been made in this investigation to define somewhat more clearly the relationship that exists between thyroid activity and the gonads. The importance of the thyroid gland on reproduction and the development of the reproductive system was studied in guinea pig and the rat. Certain observations in the course of this study have perhaps cleared some of the problems concerning a possible gonad-thyroid relationship but there is much work yet to be done before final conclusions can be established.

In studying the influence of the thyroid on the gonads it is of importance to know whether or not a sex difference can be discerned in thyroid histology. It has been impossible to note any criterion indicating sexual differences in the thyroid gland of the male or female rat or guinea pig. Gonadectomy, furthermore, has produced but little modification of the thyroid gland of either species or sex studied. In the guinea pig castration appears to produce a slight increase in thyroid weight, particularly so in the male, and histologically this appears to be due to greater storage of colloid in the follicles. In the male and female rat, however, castration produces atrophy of the thyroid acini. In the male rat, this is accentuated by a consistent loss of weight of the thyroid after castration. This is independent of the age at which the animal is operated, provided at least 50 days elapse after the operation. Thus in both guinea pigs and rats, gonadectomy results in decrease of colloid excretion into

the blood stream, in the rat, because quite obviously less colloid is produced, and in the guinea pig because very probably a greater amount of the colloid produced is stored within the follicles. The effect in the male of both species studied was far more pronounced and so physiologically speaking there does seem to exist a sex difference in the thyroid which is accentuated by gonadectomy. An added phenomenon is that gonadectomy in the rat produces a very different effect on body weight in males and females. The weight of the male rat does not change noticeably after castration whereas the female rat increases in weight considerably. It could be possible that this is correlated with the closer relationship of sexual cycle and body activity existing in the female. At any rate, only extensive studies of basal metabolism after gonadectomy can finally settle this problem.

In approaching the problem from the reverse point of view, namely the effects of the thyroid upon the reproductive system and its function, the rat alone was employed because of the more rapid reproductive rate and because thyroidectomies on certain guinea pigs showed that accessory thyroid tissue is so numerous in this species as to prohibit clear-cut thyroidectomies.

Considering first the male that has attained its reproductive capacity (70 days or older), total absence of the thyroid gland for longer periods of time appears to have but little if any direct effect upon the testicle. Males deprived of the thyroid gland for three to five months show no detectable difference in the histology of the testes from those of the littermate controls. Spermatogenesis continues actively; motile spermatozoa are almost always present in the epididymus and interstitial tissue is similar to normal controls. Smelser has reported retarded sperm production in thyroidectomized males whereas Hammett

failed to confirm this. My observations failed to indicate a reduced spermatozoon production but no volumetric counts were taken.

The seminal vesicle and the prostate gland of the thyroid-ectomized adult male rats indicate quite clearly that the testis of these animals secretes hormone in sufficient amounts to maintain the accessory organs in the histologically normal state. It cannot be definitely stated whether the amount of the hormone secreted was changed. In the majority of litters the gross weight of the seminal vesicles was less in operated than in control animals, thereby indicating a slightly lower rate of secretion after thyroidectomy, but it should be emphasized that in all cases sufficient hormone was secreted to maintain the accessory glands in a histologically normal state.

The male thyroidectomized before puberty showed a retarded development of the reproductive system. This, however, appears to be due primarily to an influence upon general metabolism and not to a specific influence of thyroid hormone directly upon the reproductive organs. Maturity as indicated by spermatozoon production and hormone secretion can be attained, even though considerably delayed, when the thyroid gland has been removed as early as the tenth day of life. No pathological changes were seen but the reproductive organs of the thyroidectomized males were retarded in their development.

In the female conditions were quite similar to those seen in the male. Removal of the thyroid from the mature female rat produced little histological modification of the ovaries or uterus. The ovaries appear to show a diminished corpus luteum content in comparison with littermate controls but sufficiently delicate methods were not employed to express this difference in

quantitative terms. The weight of the uteri of the operated animals was decreased.

Ovarian activity, however, is well indicated by the oestrous cycle. It has been stressed above that typical cycles do continue for months in the total absence of the thyroid gland. They may occur regularly but the intervals between oestrus are always considerably prolonged. A great deal of irregularity is noted and quite frequently one long dioestrous period will be followed by a shorter one.

The thyroidectomized immature female will also attain such maturity as indicated by recurring oestrous cycles but this stage, as in young males, is materially delayed.

The most convincing criteria of thyroid influence upon the reproductive system would obviously be gained from a study of reproduction itself.

It has been pointed out that many females totally deprived of thyroid for periods of two to eight months did conceive, and indicated that the number of fertilized ova was not diminished. Thus thyroidectomy does not decrease the number of maturing follicles. The state of pregnancy, however, was more seriously affected. Only a small number of these thyroidless females delivered young. These were usually not well nourished and post-natal development was poor. Many pregnant females resorbed their embryos, others delivered only after pituitrin was administered.

In thyroidectomized males my observations proved that motile spermatozoa were almost always present in the epididymis but these males failed to show any interest in females in heat and with the exception of two did not copulate. The indications are that the so-called sterility reported by others is merely due to disinclination to mate. That might be correlated with the

possible decrease of hormone produced as Döderlein's work on the guinea pig showed and vice versa active sex interest is the last sign to appear in the capon injected with male hormone. Artificial insemination would be needed to prove this point. It will be recalled that the two thyroidectomized males that did copulate each sired normal young.

There is a definite indication that there is a relationship between the luteinizing factor in the hypophysis and the thyroid. Zalesky (1934) confirmed the fact, already well known, that hypertrophy of the thyroid parallels the later stages of pregnancy when the corpora lutea are best developed. Greep (1933) maintains it is not the follicle ripening but the luteinizing fraction of the anterior pituitary that somehow activates the thyroid. The pronounced thyroid enlargement during the middle and end of pregnancy would be so explained. Another fact that corroborates the idea that a relation exists, direct or indirect, between the thyroid and the luteinizing factor of the anterior hypophysis is the normal progress of pregnancy in thyroidectomized females in the first half of pregnancy. During the second half of pregnancy which is more exclusively under the dominance of corpora lutea the embryos are frequently resorbed or die before parturition. Added evidence for a connection between the corpus luteum and the thyroid is the fact that thyroid feeding produces states of the uterus equivalent to pseudopregnancy.

The current theory as expressed by Hammett and Van Horn explains all thyroid influence on reproduction by the influence of the thyroid on general metabolism. This theory explains the phenomena observed during development of thyroidectomized animals very satisfactorily. It could also explain the changes in the sexual cycle of the thyroidectomized female after thyroid feeding

and thyroidectomy by presuming a more rapid or slower excretion of the ovarian hormones. But the depression of the thyroid after gonadectomy, slight as it might be, cannot be explained by this theory. It does seem that a hormonal influence is exerted by the gonads through the hypophysis upon the thyroid but more work will have to be done to clear this relationship.

CHAPTER V

SUMMARY

1. Gonadectomy in immature guinea pigs leads to increased thyroid colloid storage. Cryptorchid castrate males did not show this effect.

2. Gonadectomy in rats leads to slight atrophy of thyroid more pronouncedly in males but independent of the age of the animal.

3. Thyroidectomy in mature male rats produced no histological changes in the testes, seminal vesicles or prostate, but slightly decreased the weight of the seminal vesicle and frequent loss of sex interest.

4. In immature male rats thyroidectomy leads to delay in sexual maturity, correlated with retarded general body development.

5. Thyroidectomy in mature female rats decreases the number of corpora lutea in the ovary, the size of the uterus and prolongs the dioestrous period of the cycle. Young are conceived in normal numbers but the number of young born is greatly reduced by deaths or reabsorption of the fetuses.

6. Thyroidectomy in immature females leads to retarded sexual development in keeping with the generally disturbed body growth.

7. Absence of the thyroid gland produces effects upon the reproductive system that appear to be indirect rather than direct and disturbance in general metabolism appears to be responsible for the effects noted.

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